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Art. XXVI.—*On Bitter Pit and the Sensitivity of Apples to Poisons.*

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Introduction (page 367).

Part I.—The sensitivity of apples to poisons (page 368); the influence of the cuticle and bloom of apples on the absorption of poison (371); apples more sensitive than other organisms (374); do apples accumulate and permanently retain mercury from dilute solutions? (375); the actual sensitivity of the pulp cells of apples to poison (377); the influence of age (382); bases generally present in soil (384); the action of poisonous plant products (387); alkaloids (388); acids (390); the specific resistance to poisons (392); the influence of ionization and of the respective ions (394); the nature of toxicity (399); the action of gases and volative substances (400).

Part II.—The problem of bitter pit (page 404); can surface poisoning produce deep seated bitter pit tissue? (409); the starch grains of bitter pit (410); the non-solution of the starch grains (411); the source of the poisons causing bitter pit (415).

Summary (page 417).

Introduction.

In the early part of the present year, Dr. White put forward as a tentative theory the suggestion that the defect known as bitter pit in apples might be associated with the use of poisonous spraying materials for codlin moth and other pests. She was able to detect lead in the bitter pit tissue of apples from orchards which had been heavily sprayed with arsenate of lead, and from her study of the ferments of normal and affected apples, was also led to the conclusion that the defect could only be ascribed to the action of a poison. She also pointed out that bitter pit appeared to be especially common on well-tended and well-sprayed orchards, and was much less so on neglected orchards, and showed that appearances resembling bitter pit could be produced by various poisons. Curiously enough this theory, although the first one based upon any experimental evidence as distinguished from observational and imaginative evidence, met with strong disapproval, and has popularly been supposed to be entirely discredited. In the present paper I hope to be able to bring forward conclusive proof of the poisoning theory of bitter pit, and also to show that it is not necessary to withdraw or modify any part of Dr. White's paper, except in so far as her tentative theory, that the source of poisoning might be from poisonous sprays, only holds good partly, for bitter pit may also occur in orchards which have never been sprayed with poisonous compounds, although so far as the evidence goes, it appears to be much less common in such cases.

Obviously the first point of attack was to determine accurately the specific resistance of apples to different poisons. Extraordinary variations are shown in this respect by different plants. Thus among the lower organisms we have *Spirogyra* which is sensitive to concentrations of copper sulphate down to 1 part in 100 million of water, and the green mould, *Penicillium*, which can grow and thrive on fifteen to twenty per cent. solutions of the same poison. O. Richter (Die Ernährung der Algen) has shown that coins placed in mere contact with diatom cultures on agar, exercise a marked deleterious influence upon the growth of these organisms, although the coins do not lose appreciably in weight. Between these extremes intermediate grades are known, according to the specific resistance of the particular plant protoplasm, and whether it has or has not the power of preventing the penetration of the poison into its living substance.

Part I.

The Sensitivity of Apples to Poisons.

The majority of the apples used for these experiments were special ones, mainly selected ones grown in an orchard rented by me, unsprayed, and kept in the cool stores for one to five months until needed. They included the following varieties:— Jonathans, Yates' Pippin, Rokewood, Rome Beauty, Scarlet Nonpareil and Five Crown. Owing to the exhaustion of the supply before the experiments were completed, a small number of purchased apples were also used, mainly of Five Crown. These were carefully cleaned and wiped in all cases to remove any poison that might be adhering to the skin. The purest obtainable salts were used, and supplies of specially pure distilled water were obtained from the Chemical and Physiological Schools. The Minister for Customs (Mr. Tudor) kindly consented to have chemical analyses made by the Federal Analyst (Mr. P. Wilkinson), of the apples used in order to determine the amount of poison necessary to produce artificial bitter pit, and various other points connected with this question. Mr. Wilkinson's aid has been extremely valuable, and has been most zealously and ungrudgingly given.

The first tests were made with sound, uninjured apples by immersing them in solutions of mercuric chloride, copper sulphate and lead nitrate of varying strengths. In all concentrations of less than 1 in 1000, a litre of solution was used to each apple.

Copper Sulphate.—Jonathans in solution for one week, and air one week.

One gram per 200 c.c. Large and small pits from 1 cm. to a mm. diameter, and extending about half their diameter into the pulp. On submitting the material to the Federal Analyst he reported "No copper detected." In all cases the apples were well washed in running water to remove any poison adhering to the surface before forwarding them for analysis.

One per 1000. Pits smaller and less numerous.

One per 5000. None of pits exceeding 3 mm. diameter, and 1-2 mm. depth.

One per 25,000. Minute superficial spots none exceeding 1 mm. depth.

One per 100,000. No perceptible effect.

Apples in distilled water one week, and in air one week. Lenticels slightly more prominent in some cases, no other effect.

Lead Nitrate.—Jonathans.

In 1 gram per 100 cc. 1 day. After 2 weeks in air, small well-defined pits 2-4 mm. diameter, and 1 to 3 mm. deep. The Federal Analyst reported "No lead detected."

After immersal in solutions of 1 per 1000, 1 per 5000 and 1 per 10,000 for one week, the apples showed in five weeks a few small spots on the surface not exceeding 1 mm. diameter and less than a millimetre depth, but no distinct pits.

With solutions of 1 gram in 25,000 and in 1,000,000 c.c. of water, no spots or pits at all appeared, even after one week's immersal, and subsequent keeping in air for six weeks.

In order to determine whether penetration was favoured by changes of temperature such as occur under natural conditions, apples were heated to 40 deg. C, daily for a week, and allowed to cool under a solution of 1 gram per 1000 c.c. Much air escaped from the lenticels (breathing pores), but only a few small pits developed under some of these, the largest not exceeding 3 mm. diameter after three weeks in air. If the temperature rises above 40 deg. C, the surface layer is apt to be scalded, but evidently changes of temperature do favour the entry of poison, but not as much as might be expected, and only through the lenticels.

Mercuric Chloride.—Jonathans.

In one gram per 100 cc. for one day, after one week in air, 28 large and small pits present, in 7 grams per 1000 after the same times, 29 mostly smaller pits developed. After immersal in 4 per 1000 solution for two days and one week in air, 13 small pits formed, and after the same times with 2 per 1000, three very small pits. After one week in 1 per 5000 and two weeks in air, several spots and seven small pits, the largest 3 mm. diameter appeared. The whole of these apples (5 experiments) were separately tested by the Federal Analyst, who reported that "the whole of the pitted portions in each apple (the peel was removed previously by a knife) was used for the test, no indication of the presence of a mercury compound was detected in any instance, and the method of analysis used was sufficiently delicate to detect the 1-100,000th part of one grain of mercury."

These results seem to show (1) that the sound skin of the apple is very impermeable to soluble poisons, (2) that penetration is possible through the breathing pores producing pits confined to the point of entry, and (3) the amount of poison so penetrating is extremely small. They also show that the fact that the State Analyst was unable to detect any lead or arsenic in the limited quantity of bitter pit apples collected by the

State Committee from unsprayed orchards did not dispose of the poisoning theory of bitter pit, as was naturally supposed to be the case by those unaware of the facts known by the plant physiologist in regard to oligodynamic poisoning.

The influence of the cuticle and bloom of apples on the absorption of poison.

Sound, unhandled Five Crown apples with a perfect bloom were (a) wiped with a rough cloth on one side to remove the bloom, and (b) had an equatorial band of bloom scraped off with a razor without injuring the skin, and were floated for 1 week in a solution of 2.5 grams of copper sulphate ($CuSO_4 . 5H_2O$) in 1000 c.c. of water. Examined a week later (a) showed scattered spots and small pits, 1 mm. diameter and depth on the wiped side, and a few minute spots on the unwiped side, whereas (b) showed a faint brown line joining an equatorial row of pits, varying from 1 to 5 mm. diameter, and from superficial to 4 mm. depth. Close examination showed, however, that the largest pits were under breathing pores, whose orifices had been enlarged by the scraping.

A similar test was performed with a solution of 1 gram of mercuric chloride in 1000 c.c. of water. The wiped side was browned and irregularly pitted, while the unwiped side was very faintly browned, but the surface remained smooth and round with several minute spots, but no pits.

To obtain additional evidence of the influence of the bloom, 15 Jonathan apples with a perfect bloom were selected, and without handling them the calyx and stalk were closed with melted paraffin. They were completely immersed in a 1 per cent. solution of mercuric chloride for one day, and then well washed. In one week they were covered with large and small bitter pits mainly confined to the breathing pores, but the general pulp remained sound, and the surface was barely or not at all discoloured, apart from the pits. They were then forwarded for analysis. The Federal Analyst reported, "The diseased pulp and skins were separately analysed. I did not succeed in detecting the presence of mercury. The procedure followed, from comparative trials with known quantities, would enable the presence of approximately 1-10,000th of a grain of mercury to be detected."

The waxy bloom was removed from a further set of 15 Jonathans which were immersed for a week in a half per cent. solution of mercuric chloride, allowing them to float and aerate themselves for one hour daily. The apples all had sound, unbroken skins, large stalks and well-closed calyces. They were covered with large dead brown patches, extending in some cases nearly to the core, and there was evidently a slight penetration at the calyx end, and still more at the stalk end of the fruit. Hence after thorough washing, a one centimetre cylinder of the core was removed, and the remainder forwarded for analysis.

The report from the Federal Analyst was as follows:—"The fifteen samples of apples referred to in the attached letter from Professor Ewart have been examined as to the presence of mercury compounds as detailed hereunder:—

A.	Mercury on the exterior surface of the apples (as removed by chlorine and washing) calculated as mercuric chloride	17 mg.
B.	Mercury contained in thin peelings, calculated as mercuric chloride	20 ,,
C.	Mercury contained in brown pit pulp, outer layers, calc. as mercuric chloride	16 ,,
D.	Mercury contained in deeper layers of pit pulp, calc. as mercuric chloride	18 ,,

"The quantities of mercury reported are probably, owing to imperfections in method of determination, slightly below the amounts actually present in the apples."

In this experiment the total volume of the solution was 2000 c.c. and of the apples 2760 c.c. The weight of mercuric chloride in the solution was 10 grams, and the weight of mercury found in the apples 71 milligrams, representing 96 milligrams actually absorbed of the chloride, or rather more than this allowing for losses in analysis. Hence the concentration in the apples after a week's immersal did not exceed 1-100th of that of the solution outside.

An exactly similar experiment was performed with 12 wiped Jonathan apples, but the calyx and stalk were covered with paraffin, and the time of immersal shortened to three and a-half days. The whole surface was affected, and more or less pitted and wrinkled. In one week the whole superficial pulp was browned in spots to a depth of 1 to over 10 cms. The Federal Analyst reported that "the quantity of mercury compounds recovered from the peel of the twelve apples was 0.036 gramme (as metallic mercury) and from the brown-coloured spots of the pulp 0.009 gramme. The amount of mercury recovered is considered to be less than that actually present." This is because of the relatively large bulk of organic material, which might in extreme cases cause reductions in the apparent amounts present, up to as much as 20 or even 25 per cent. Even then the total amount of mercuric chloride entering the apples would not exceed 0.06 gram, and that in the pulp 0.012 gram. This was spread over not more than one-third of the 1890 c.c. bulk of apples. Hence the concentration in the dead pulp after three and a-half days could not have been more than 1 in 5000, or 1-25th of that in the liquid outside.

A third experiment similar to the preceding one was performed with four wiped Jonathans, immersing them for three days in a 1 per 1000 solution of mercuric chloride. After well washing and keeping in air for 14 days, the whole surface was brown, discoloured and irregularly pitted, with brown pulp from 2 to 20 millimetres deep in parts. From these apples the Federal Analyst recovered 0.004 gramme as metallic mercury from the peel and pulp together. The apples had an original volume of 615 cc. The two litres of solution used contained 2000 milligrams, of which only four were absorbed by the apples. Here, as in the previous experiment, the concentration within the apple was very considerably less than that outside, and the concentration of poison in the deeper layers of dead pulp must have been extremly small.

Additional evidence of the influence of a waxy bloom in preventing the entry of poison was obtained by painting apples over rapidly with a very thin coat of low melting point paraffin. At a casual glance these apples showed nothing abnormal in appearance. Close examination was necessary to detect the treatment they had undergone. They were floated on half per cent. solutions of mercuric chloride, copper sulphate and lead nitrate for one week, and the artificial bloom then removed by wiping with a cloth and warm water. There were no signs of asphyxiation in this time, in spite of the blocking of the pores by paraffin, but the apples developed a slight alcoholic smell. After three weeks in air, all the apples remained quite sound, with no signs of any development of bitter pits superficial or deep-seated.

Apples more sensitive than other organisms.

All the poisoned apples from these and the following experiments[1], which were not used for analysis or microscopic examination, were fed to guinea pigs, and these included apples soaked in half per cent. solutions of mercuric chloride and copper sulphate, and then kept until a large part of the pulp was dead and brown. The guinea pigs, beyond occasionally showing thirst and micturating freely, showed no signs of poisoning, but they usually refused to eat the skin and worst rotted parts of the most strongly poisoned apples. In any case, in the course of several months each guinea pig ate at least twice its own bulk of poisoned apple pulp without suffering appreciably.

In addition, wiped apples were soaked in 0.5, 0.2 and 0.1 per cent. solutions of mercuric chloride, and in 1 and 5 per cent. solutions of copper sulphate for one week, and then kept until the surface pulp was rotted wholly or in part. The skin was then broken in places and infected with the spores of the green mould, *Penicillium.* The hyphae developed in the dead pulp nearly as rapidly as in dead unpoisoned apples killed by boiling, and more rapidly than in the pulp of sound apples. Spores were also formed freely, mainly from the broken surface of the skin. From the analyses given above, the percentage of mercury in the dead pulp cells could not have greatly exceeded 1 in 10,000, and since *Penicillium* can develop well on solutions containing as much as 15 per cent. of copper sulphate, it is not surprising that it should be more resistant to mercury than the pulp cells of the apple. Possibly its hyphae may be more or less impermeable to soluble mercury salts just as they are to copper salts,

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1 Excepting those treated with alkaloids.

for since mercury is a general protoplasmic poison, once it enters living protoplasm in a soluble form it cannot fail to exercise a poisonous action.

Do Apples accumulate and permanently retain Mercury from dilute solutions?

To determine this, six peeled quartered Jonathans, having a total volume of 820 c.c., were immersed in 6 litres of a 1 per 10,000 solution of mercuric chloride for one week until browned right through. The fact that the browning took place in the presence of mercuric chloride seems to show that it is not the result of the action of an oxidase ferment, but is the result of direct oxidation upon substances which either come into contact when the cell is killed or are protected from oxidation while it is alive.

The Federal Analyst reported that "the sample submitted for analysis has been found to contain approximately 50 milligrams of mercury compounds, calculated as metallic mercury. For the total bulk this represents 204 milligrams of mercuric chloride not removed by washing, but held in more or less permanent combination."

The residual solution, after the apples had been removed, was too dilute to test for mercury by volumetric means. It turned slightly brown with sulphuretted hydrogen, but formed no precipitate; it gave no opalescence with ammonia, and there was no perceptible change of colour when an excess was added to a 1000th normal solution of potassium iodide. Hence, to determine the amount of mercury present, a clean strip of pure copper was immersed in 1000 c.c. of the liquid. It took three weeks before all the mercury was deposited. The copper strips were weighed before and after heating in an atmosphere of hydrogen, the decrease in weight giving the amount of mercury deposited. One litre contained 38 milligrams of mercury, or 47 of mercuric chloride. Hence the apples had apparently absorbed approximately one half of the total quantity of poison; that is, 318 milligrams or 388 milligrams per litre; that is, three to four times the original concentration in the liquid outside. Hence the pulp cells of apples are able to accumulate such metallic poisons as mercury in greater concentration than in a dilute solution presented to them.

Additional evidence was obtained in the following way:—Two cubic centimetres of the pulp of two of the above apples were removed, and, after washing, placed in one litre of distilled water with a prepared Rome Beauty floating on top. The liquid was gently stirred daily, and after one week the apple was removed. The prepared spots already showed signs of poisoning, and after one week in air developed from a superficial browning to pits 1 millimetre deep. The 2 centimetres of poisoned pulp were then crushed, and kept in contact with a clean strip of pure copper for one week. No trace of mercury was deposited. The pulp was then treated with sulphuric acid, nearly neutralised, and kept in contact with a clean strip of copper for one week. A slight but distinct stain of mercury was formed, volatilising on heating, but not in sufficient quantity to be accurately weighable. Judging from a comparative test the total amount was a very small fraction of a milligram.

It follows therefore that the pulp cells do fix mercury, and that the mercury is liberated again by treatment with sulphuric acid. The poison that diffused from the dead pulp would at first be merely mercuric chloride in solution. Since the concentration outside was 388 milligrams per litre, not more than 0.7 of a milligram of mercuric chloride could have been present in solution in the poisoned pulp. This would produce at the most a concentration of 7 in 10,000,000 in the litre of water in which the prepared apple lay, and probably considerably less, since the poison as it exudes is being absorbed by the second apple. The special interest of this observation lies in the fact that if its rationale were not known, it might have led to the conclusion that the dead pulp of one apple was able to communicate a disease to the second one. Instances of oligodynamic poisoning may be much commoner than is generally supposed, and it is in fact possible that certain other diseases of plants and even animals may directly or indirectly have a similar origin. All that is necessary is to have sensitive cells capable of absorbing or accumulating very minute traces of poison. If these cells were in a growing tissue the symptom of poisoning might be an increased or abnormal growth, and if this kept pace with the accumulation of poison the latter might remain in an almost infinitesimal concentration, and yet produce a pronounced external or internal effect. This is, of course, a suggestion only, but one that seems worthy of consideration, particularly in regard to certain abnormal or cancerous growths, not due to specific disease organisms.

Hypodermic injections of poison.

In order to determine the actual sensitivity of the pulp cells to poison, it is necessary to be sure that the poison penetrates freely. Hence experiments were made by using a fine hypodermic syringe to inject small amounts of very dilute solutions of poison into the pulp of sound apples. Blank injections and injections with tap water and distilled water produced even after six weeks no spreading from the point or line of puncture (the needle and liquid were sterilised before each puncture), but the surface became slightly depressed around each puncture, owing to the increased evaporation where the cuticle was broken. Injections of approximately one-tenth of a cc. of 1 per 100,000 solutions of copper, mercury and lead after six weeks looked superficially no different to blank injections, but except in a few cases where the needle evidently became blocked in puncturing, well-developed bitter pit areas were present around or at the base of the punctures having a diameter of 3 to 5 mm., and in the largest of these areas a few cells packed with starch grains were found.

However carefully the puncturing is done, the loose apple pulp cells block the needle more or less, so that it is impossible to inject accurately measured quantities. Owing to the physical differences between apple pulp and animal tissue, this method is in fact not suitable for exact quantitative investigations in this particular case, and hence was abandoned in favour of a more exact and controllable *modus operandi.*

The actual sensitivity of the pulp cells of apples to poison.

The method used was to remove around the equator of each apple fragments of the cuticle approximately a millimetre square at intervals of a centimetre, by means of a clean, sharp razor, and to float the apples in a litre of the dilute solution to be tested. Apples almost always float with their equatorial line submerged and horizontal. Controls were performed with each set of experiments. It was found with practice easy to remove fragments of the cuticle without injuring the cells beneath the epidermis. These fragments must be quite colourless and transparent. If they show any colour some of the subjacent cellular tissue has been removed. Such apples are denoted in the following pages as " prepared " apples, and very close examination with a lens is necessary to detect the fact that they have been prepared. If kept in moist air, the prepared spots undergo no change or sinking, and are unaffected even by a week's immersal in pure distilled water. Occasionally, however, and particularly in the case of Five Crown apples, wet rot may start at a prepared spot, but this is quite different from the bitter pit change, which is always ultimately characterised by dark-coloured or brown, dry, more or less shrivelled cells with brittle walls, even when produced by long immersal in a dilute poisonous solution. The pits are, in fact, often noticeably dry when the apples are taken out of the poisonous solution, and always become so after it has been kept in air. In wet rot, however, whether due to fungal hyphae or not, the tissue always seems to become soft and watery.

Owing to the exhaustion of the supply of unsprayed apples from my own orchard, some of the later tests were performed with purchased apples whose history was unknown, but which had in many cases obviously been sprayed. These were cleaned with a cloth and warm water, but this was not always sufficient to remove the poison adhering to the skin. In such cases the controls in water may show a well-developed pit under one or more of the prepared spots while the others are quite unaffected, and the results with increasing dilutions of poison will show more or less pronounced inconsistencies. This difficulty was most pronounced with the Five Crown apples, which also seem to be more affected by submersal in water than the other kinds used. In all such cases the experiments were repeated, and they are only given when consistent results were obtained with the first, second and even third trials. On this account shorter periods of immersal were used with the Five Crowns sorts. Apart from the necessity of using pure copper-free distilled water, care must be taken that the glass vessels used are chemically clean. The best method of assuring this was found to be by polishing them internally (1½ litre gas cylinders) with a damp cloth and precipitated chalk, and then rinsing well with tap water, and finally with distilled water. This method was as effective as coating the vessels internally with paraffin. A litre of solution was used to each apple, since this appears to be approximately equal to the total quantity of water used or stored by a fairly large apple during its development while attached to the tree.

Mercuric Chloride.—

Prepared Scarlet Nonpareils.—Floating on solution one week, and examined after three days in air. One gram $HgCl_2$ per 200,000 c.c. water. Large, deep brown pits at each prepared spot.

One gram per 1,000,000 of water. Pits similar, but not quite so deep. The Federal Analyst reported that "extremely faint but distinct indications of mercury were detected in the form of metallic mercury. The quantity present in each instance was less than approximately 1-100,000th part of one grain." The total bulks of "diseased" tissue were approximately 2 and 5 c.c. Hence the concentration in the affected tissue lay between one in 30 millions and one in 75 millions, i.e., very considerably less than in the solution outside. The poison has, however, to diffuse in through very narrow points of entry, and then spreads in the internal pulp in concentric hemispheres of decreasing concentration as far as the pit extends, so that at the outer margins the concentration will be much less than at the point of entry.

Prepared Jonathans.—Immersed one week. Examined after one week in air.

One per 10,000. Large spots extending deep into pulp, and some confluent.

One per 100,000. Pits smaller and each one distinct.

One per 500,000. Deep, well developed pits to each prepared spot.

One per 2,500,000. Pits not so deep or broad.

One per 25,000,000. Pits from 2 to 6 mm. diameter, and 2 to 4 mm. deep beneath each prepared spot.

Immersed one week and examined after two weeks in air.

One per 50,000,000. Distinct brown pits from 1-3 mm. deep beneath each prepared spot.

One per 100,000,000. Same as preceding, but pits a little fainter and shallower, and some spots showing a little superficial browning only.

One per 1,000,000,000. Three pits 2 mm. deep, two of prepared spots with a brown rim, rest no signs of poisoning.

One per 10,000,000,000. One prepared spot with faint brown rim, rest unaffected.

One per 100,000,000,000. No trace of any poisonous action.

In pure copper-free, distilled water, no diseased tissue at the prepared spots, pits or marks of any kind.

Copper Sulphate.—

Prepared Jonathans.—One week in solution, and examined after a week in air.

One gram in 100,000 c.c. (250 cc. of solution). Well developed pits up to ½ cm. diameter, and mostly 2-3 mm. deep.

One per 200,000. Pits well developed. The Federal Analyst reported, "No copper detected."

One per 500,000. Pits well developed, some nearly 5 mm. deep.

One per 1,000,000. Pits less developed, but some 3 to 5 mm. deep. The Federal Analyst reported, "No copper detected."

One per 2,500,000. Pits beneath each prepared spot, but none exceeding 2-3 mm. deep.

One per 25,000,000. Superficial browning to pits with dead tissue, not exceeding 1 mm. beneath most of the prepared spots.

One per 100,000,000. Slight superficial browning at some of the prepared spots.

One per 1,000,000,000. No signs of any poisoning.

Hence, although the pulp cells of apples are extraordinarily sensitive to copper sulphate, they are less so than to mercuric chloride. Nevertheless the traces of copper sometimes present in distilled water are sufficient to affect them. Thus a prepared apple was floated on 250 c.c. of water distilled in a copper vessel and tubes. The water was renewed daily for one week, and after two weeks in air distinct pits up to 3 mm. depth had developed beneath all the prepared spots. Similar apples on 100 c.c. of the same water for one week and on water distilled in glass vessels developed no perceptible pits.

All varieties are not equally sensitive, and Rome Beauties are, for instance, distinctly less sensitive than Jonathans. Thus after similar treatment and times on a solution of 1 gram per million of water, the pits were more superficial and not more than 1 to 2 mm. deep, and no trace of any poisonous action on this variety could be detected with solutions of 1 per 100 millions. There is, however, a possibility that the conditions under which an apple has been grown may affect its resistance to poison, and actual evidence of this is given later, so that apparent differences between varieties may not be as prominent in some cases as in others.

Lead Nitrate.—

Prepared Rokewoods.—One week in 1 litre of solution, and examined after two weeks in air.

One per 10,000,000. Pits 1-3 mm. deep beneath each prepared spot.

One per 100,000,000. From no effect to slight superficial browning.

One per 1,000,000,000. No perceptible signs of poisoning.

Prepared Jonathans.—One week in 1 litre of solution, and examined two weeks later.

One per 200,000. Pits from superficial to 5 mm. deep. The Federal Analyst reported, " No lead detected."

One per 1,000,000. Distinct pits up to 2 or 3 mm. deep under some, but not all of prepared spots. The Federal Analyst reported, " No lead detected."

Lead nitrate is evidently distinctly less poisonous in its action than mercuric chloride, and is slower in its action and penetration than the sulphate of copper. In the following tests with prepared Jonathans, after one week's immersal the apples were kept in air for nine weeks[1].

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1 In all cases the apples after washing were kept in the same cleaned gas cylinders in which they had been tested. These keep the apples well aerated and yet in moist air without danger of infection. They keep for a remarkably long time under such circumstances without shrivelling or rotting.

One per 100,000 (250 c.c. of solution)[1]. Well developed, but small pits, none exceeding 3-4 mm. diameter and 1-2 mm. depth. Numerous minute black spots on skin, but the pulp quite sound.

One per 500,000. As above, but no spots on the skin.

One per 2,500,000. Pits barely exceeding 1 mm. depth, and not spreading laterally beyond the prepared spots.

One per 25,000,000. From superficial browning to a few pits 1 mm. deep.

One per 100,000,000. Superficial browning on most, but not all, of the prepared spots.

One per 1,000,000,000. No signs of any poisoning action on any of the prepared spots.

One point specially noticeable is, that with very dilute solutions, the quantity of solution is as important as its concentration. Thus a litre of 1 per 500,000 exercises the same action as 250 c.c. of 1 per 100,000, and a litre of 1 per 200,000, which contains twice as much poison as 250 c.c. of 1 per 100,000 also exercises a greater poisonous action when the same area of apple pulp is exposed to it for a sufficient length of time.

All varieties are not equally sensitive. Thus prepared Rome Beauties kept in the solution one week, and examined after two weeks, showed only superficial browning to pits under 1 millimetre deep in a solution of 1 per million, and no perceptible effect in a solution of 1 in 10 millions.

The influence of age.

In order to determine the influence of age upon the sensitivity to poison, young Jonathans about three weeks old were selected, averaging 3 cm. in length by 2 cm. in diameter. They were immersed in solutions of lead nitrate for one week, and after being kept in moist air for a week were examined.

Apples with the normal uninjured surface gave the following results with lead nitrate: —

One per 1000. Numerous small pits with irregular edges, mainly superficial, and none exceeding 1 mm. deep and 2 mm. diameter.

1 In all cases where not otherwise stated, 1 litre of the solution was used to each apple, which approximates to the amount of water an apple of 100 c.c. bulk may absorb from the tree during its development.

One per 10,000. From none to several mainly superficial pits, none exceeding ½ mm. deep and 1 mm. diameter.

One per 100,000. No perceptible effect.

At this stage apples have the cuticle very feebly developed, the bloom absent, and the minute temporary breathing pores (stomata) have not yet been replaced by the larger adult ones (lenticels). The skin is therefore more or less generally permeable to poison, and the pits are developed without any relation to breathing pores. The permeability of the skin is also shown by the fact that in fairly dry air the apples after picking undergo a pronounced loss of weight in one day, and in three to seven days begin to shrivel.

With prepared apples the young pulp cells showed themselves to be very much more resistant than the pulp cells of adult apples.

One per 10,000. Strongly marked but mainly superficial pits beneath each prepared spot, none exceeding 2 mm. diameter and 1 mm. depth.

One per 100,000. Superficial layers browned and killed at each prepared spot, but no distinct pit formation.

One per 1,000,000. From no perceptible effect to distinct but entirely superficial browning (two to three layers of cells brown and dead).

One per 10,000,000. No perceptible effect.

In dry air the prepared areas show a tendency to cork formation, but not in water or in moist air. These results show that while in these young apples the skin is more readily permeable than in adult ones, the pulp cells are some hundred times more resistant to such a poison as lead nitrate. Hence sufficient poison might enter a young apple to produce internal poisoning when adult, without showing any signs of its presence at first, beyond hindering or preventing the solution of deposited starch grains. Later on the affected cells would die, not so much because the poison increased in amount as because the resistance of the cells decreased with age. It is worthy of note in this connection that these young Jonathan apples three or so weeks old contain plenty of active chloroplastids particularly in the outer layers, but no starch grains either in the healthy pulp or superficial cells, or in the poisoned pit cells.

The starch grains are deposited at a later period and then dissolved during ripening.

Bases generally present in soils.

Although the foregoing metallic poisons are found in certain soils they are usually of limited occurrence, although in the soils of orchards that have been frequently and freely sprayed with poisonous metallic compounds, appreciable quantities may be retained in the soil. As is well known, fertile soils have a pronounced retentive action upon many metallic poisons, and particularly those which like mercury combine with organic matter (proteids, etc.). Nevertheless an abundant but feebly poisonous base can exercise the same effect as a scarce but highly poisonous one if absorbed in sufficient amount.

The bases used were magnesium, calcium, barium and potassium, as the sulphates and chlorates.

Magnesium Sulphate.—

Prepared Five Crowns.—Immersed three and a-half days in solution, and examined after 14 days in air.

One per 1000. Pits 3-12 mm. diameter; 2-5 mm. deep.

One per 10,000. Pits 2-5 mm. diameter; 2-3 mm. deep.

One per 100,000. Pits 2-3 mm. diameter; 1-2 mm. deep

One per 1,000,000. Slight superficial browning only.

One per 10,000,000. No perceptible poisoning effect.

Zinc sulphate gave practically identical results, except that the poisoning effect was more distinct with 1 per 1,000,000, and was perceptible in this dilution even with Rome Beauties.

This sensitivity was quite unexpected in the case of magnesium sulphate, but the apples were purchased ones of unknown history, and Five Crowns appear to be somewhat sensitive to prolonged immersal in water. Another batch, however, gave closely similar results, but other varieties were much less sensitive.

Prepared Rome Beauties.—One week in solution and examined after one week in air.

One per 1000. From superficial browning to pits 2 mm. deep.

One per 10,000. From superficial browning to pits 0.5 mm. deep.

One per 100,000. No perceptible poisoning effect.

Calcium sulphate gave exactly similar results to magnesium sulphate with Rome Beauty apples.

Barium Chlorate.—

Prepared Jonathans.—In solution for one week, and examined after two weeks in air.

One per 10,000. Pits 1-4 mm. diameter and 1-3 mm. deep.

One per 100,000. From superficial browning to pits 1 mm. deep.

One per 1,000,000. No perceptible development of pits or browning.

Prepared Rome Beauty.—One week in solution, one week in air.

One per 1000. Dark, well-developed pits 1-3 mm. deep.

One per 10,000. From superficial browning to pits 1 mm. deep.

One per 100,000. From slight superficial browning to no effect.

One per 500,000. No perceptible poisoning action.

Potassium Chlorate.—

Prepared Jonathans.—One week in solution, and examined after two weeks in air.

One per 10,000. From superficial browning to slight pit formation, none exceeding 1 mm. deep.

One per 100,000. No perceptible poisoning action.

Prepared Rome Beauty.—In solution one week and air one week.

One per 1000. From superficial browning to pits 2 mm. deep.

One per 10,000. From slight superficial browning to pits 0.5 mm. deep.

One per 50,000. No perceptible poisoning action.

Of these four salts the barium one is most poisonous[1], the magnesium and calcium next, while potassium is one of the least poisonous. The last three are all essential elements of the plant's food, but as we shall see later they do not exercise the same poisonous action when they are present jointly in the proper proportions, as when present singly. In addition, with the

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1 Mr. Wilkinson informs me that barium is more commonly present in soils than is generally supposed.

more dilute solutions, symptoms of poisoning (browning of cells, etc.) usually do not show until after the removal from the solutions, and even although the apples are kept in moist air, there is a possibility of some concentration taking place. This is, however, just what occurs under natural conditions whenever a plant absorbs any substance which it does not use, or does not use as rapidly as it absorbs it, both of which cases are of common occurrence in nature. In any case the artificial bitter pit tissue has the same peculiar dry, corky appearance of natural bitter pit, whether it is produced by poisonous heavy metals or metals of the alkali and alkaline earth groups, and whether it is produced in a watery solution or not. Mere superficial examination is sufficient to distinguish it from the soft brown rot produced by fungi, strong doses of poison, etc. The browning of the tissue, the brittle cell walls and the tendency to shrinkage and cracking are not shown when the pits are produced by relatively strong doses of acid, but are when very dilute solutions are used, i.e., when the passage from life to death is gradual instead of sudden. Hence the appearance of certain features of bitter pit is rather a characteristic of life than of death. If a peeled apple is soaked in a solution of 1 per 100,000 of mercuric chloride, a brown zone travels slowly inwards, preceded by a pale zone a few millimetres broad. In a 1 per 10,000 solution, the pale zone is much narrower, and contains living cells at its inner border, whereas in the browned tissue all are dead. The brown colour is evidently not due to any ferment action, or it would not be produced in the presence of mercury, but is possibly the result of the direct oxidation of a chromogen produced or liberated during the death of the pulp cells.

Although gold is never present in great abundance, traces of it in soluble form occur in the sea, and possibly also in some soils and in soil waters.

Gold Chloride.—

Prepared Yates' Pippin.—Four days in solution, and examined after one week in air.

One per 10,000. Pits 2 to 5 mm. deep.

One per 100,000. Pits 1 to 2 mm. deep.

One per 1,000,000. Pits superficial to 1 mm. deep.

One per 10,000,000. Superficial browning only.

One per 100,000,000. No perceptible signs of poisoning.

One per 1,000,000,000. No perceptible signs of poisoning.

In the 1 per 10,000 solution the contact with organic matter and the presence of light causes gold to be deposited, the liquid becoming golden in reflected, and green in transmitted, light. In the 1 per 100,000 the gold separates in a still finer state of subdivision, the liquid becoming a clear purple colour, barely perceptible in the 1 per 1,000,000 solution. Gold is deposited on the skin of an apple in a 1 per 10,000 solution, and can be removed by rubbing with a cloth. In the 1 per 10,000 and the 1 per 100,000 solutions the prepared spots are black on the surface, and a deeper brown than usual within. This decomposition of the gold chloride at the surface may explain why the pulp cells appear to be so much less sensitive to gold chloride than to mercuric chloride or copper sulphate.

The action of poisonous plant products.

Since formaldehyde is one of the primary products of the photo-synthesis of carbon dioxide, and since young apples contain chloroplastids capable of active photo-synthesis, a fact which was determined by means of the Bacterium method, the possibility needed consideration of a self-poisoning by the continued production of formaldehyde, while its polymerization to sugar or starch was for some reason suppressed.

Sound adult Scarlet Nonpareil apples, which had been for three months in cool storage were floated on a 1 per cent. solution (1 c.c. of 40 per cent. in 100 c.c. of water) for one week. They were covered with dark, irregularly distributed sunken areas, but even after three weeks the marks were superficial, and did not extend into the pulp. No trace of formaldehyde could be detected in them, the poison, being volatile, having long since evaporated. In a 1 per 1000 solution, numerous small spots appeared, and in a 1 per 10,000 a few minute ones, but these were also entirely superficial. Solutions of 1 in 20,000 and 1 in 100,000 produced no effect.

Formaldehyde.—

Prepared Yates' Pippin.—Three days in solution, and examined in three days.

One c.c. of 30 per cent. solution in 100 of water. Pits from 5-6 mm. deep.

One per 1000. Pits 4-5 mm. deep.

One per 10,000. Pits superficial to over 1 mm. deep.

One per 100,000. No perceptible signs of poisoning.

Apples are therefore very much less sensitive to formaldehyde than might have been expected, and a relatively considerable accumulation of it would be necessary to poison the pulp cells. Since it is a volatile substance it could hardly produce a permanent arrest of ferment action on the starch grains. The same objection applies to the following alkaloids, which are produced by some plants, but not by apples.

Alkaloids.

To many plants, and more especially to those which produce them, alkaloids are comparatively non-poisonous, and some fungi may even use them as food. Generally speaking, the alkaloids are rather nerve and muscle poisons than protoplasmic poisons, and a plant which has no nerves or muscles may or may not be sensitive to alkaloids, which are deadly poisonous to warm-blooded animals. Strychnine appears to be the most poisonous of the alkaloids to those plants which are sensitive to alkaloids. In this case apples appeared to show a quite unusual and unexpected general sensitivity to poisonous alkaloids.

Brucine Nitrate.[1]—

Prepared Five Crown apples.—Three and a-half days in solution, examined after one week in air.

One per 1000. Pits ½ to 1 cm. diameter, and 2 to 5 mm. deep.

One per 10,000. Pits ½ to 1 cm. diameter, and 1 to 4 mm. deep.

One per 100,000 Pits 2 to 5 mm. diameter, and 1 to 3 mm. deep.

One per 1,000,000. Pits 1 to 2 mm. diameter, and ½ to 1 mm. deep.

One per 10,000,000. No perceptible poisoning action.

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1 Brucine nitrate dissolved in very dilute HNO_3 and solution neutralized with NH_4HO If the acid is sufficiently dilute, no brownish red colour is produced.

One per 100,000,000. No perceptible effect.

Morphine sulphate gave exactly similar results to the above.

With strychnine sulphate the following results were obtained:—

One per 1000. Pits ½ to 1 cm. diameter, and 2 to 5 mm. deep.

One per 10,000. Pits ½ to 1 cm. diameter, and 2 to 4 mm. deep.

One per 100,00. Pits 2 to 5 mm. diameter, and 1 to 3 mm. deep.

One per 100,000. Pits 2 to 5 mm. diameter, and 1 to 3 mm. deep.

One per 10,000,000. No perceptible poisoning action.

Similar results to those with strychnine sulphate were obtained with atropine sulphate.

If the apples are used, as these were, late in the season, it is necessary that they should not be kept long after removal from cool storage before testing. Otherwise the pulp cells have so delicately balanced a vitality that imperceptible or "spontaneous" causes may upset the balance, and in such cases it is difficult to obtain regularly consistent results obviously due to the poison used.

The following tests were performed with Rome Beauties freshly taken from cool storage, and after washing prepared as usual for testing by the removal of square millimetres of cuticle a centimetre apart around the equators of the apples. The apples were floated for one week in the solution, and kept for one week in air before examination.

Brucine Nitrate.—

One per 10,000. Pits 1 to 2 mm. deep.

One per 100,000. Superficial browning to pits 1 mm. deep.

One per 1,000,000. No effect to slight superficial browning.

One per 10,000,000. No perceptible effect.

Strychnine Sulphate.—

One per 100,000. Pits 1 to 2 mm. deep.

One per 1,000,000. From superficial browning to pits barely exceeding 0.5 mm. in depth.

One per 10,000,000. No perceptible poisoning action.

Apparently therefore Five Crown apples are more sensitive to alkaloids than Rome Beauties, but strychnine sulphate and morphine sulphate are relatively more poisonous to Rome Beauties as compared with brucine nitrate than to Five Crowns.

The sensitivity of different organisms to strychnine sulphate varies greatly. Thus Andrews[1] found that a concentration of 1 per 100,000 rapidly kills *Volvox*, whereas a concentration of 1 per 1,000,000 left *Diatoms*, *Desmids*, and blue green *Algae* unaffected, and a solution of 1 in 250 was required to kill ordinary fresh water and marine *Algae.*

Acids.

The commonest acid produced by plants apart from carbonic acid is perhaps oxalic acid. but citric acid may be produced by certain fungi and by fruits, nitric acid may be produced by nitrifying soil bacteria, and sulphuric acid by certain sulphur bacteria.

Oxalic Acid.—

Prepared Five Crown apples.—Three and a-half days in solution, and examined after one week in air.

One per 1000. Pits 4 to 12 mm. diameter, and 3 to 6 mm. deep.

One per 10,000. Pits 3 to 8 mm. diameter, and 2 to 4 mm. deep.

One per 100,000. Pits 1 to 4 mm. diameter, and 1 to 2 mm. deep.

One per 1,000,000. From no perceptible effect to superficial browning.

One per 10,000,000. No perceptible effect.

Prepared Rome Beauty.—One week in solution, and examined after one week in air.

One per 10,000. Pits 1-3 mm. deep.

One per 100,000. From superficial browning to pits under 1 mm. deep.

One per 1,000,000. From no effect to slight superficial browning.

One per 5,000,000. No perceptible effect.

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1. Proc. of the Indiana Academy of Science, 1905, p. 195.

Citric Acid.—

Prepared Rokewood Apples.—In solution one week, and examined after one week.

One per 10 000. Pits 1 to 4 mm. deep.

One per 100,000. From superficial browning to pits 1 mm. deep.

One per 1,000,000. No perceptible effect.

Prepared Rome Beauty Apples.—In solution one week, and examined after one week.

One per 10,000. Small pits, few exceeding 1 to 3 mm. deep.

One per 100,000. From superficial browning to pits 1 mm. deep.

One per 1,000,000,000. No perceptible effect.

Nitric Acid.—

Prepared Five Crown.—Three days in solution, and examined after one week in air.

One per 10,000. Large deep pits to each prepared spot.

One per 100,000. Pits under 5 mm. diameter and 3 mm. depth.

One per 1,000,000. Pits small, superficial, none over 1 mm. deep.

One per 10,000,000. Superficial browning only.

One per 100,000,000. Doubtful to no perceptible effect.

One per 1,000,000,000. Doubtful to no perceptible effect.

Prepared Rome Beauty.—One week in solution and one week in air.

One per 10,000. Pits 1-3 mm. deep.

One per 100,000. Superficial browning to pits 1 mm. deep.

One per 1,000,000. Doubtful to no perceptible effect.

One per 10,000,000. Doubtful to no perceptible effect.

These apples were wiped and had a good bloom, but a few small brown spots appeared on the general surface with a concentration of 1 per 10,000, none with lesser concentrations. An apple with the bloom scraped off from a line around the circumference showed a line of pits up to 5 mm. deep, but only beneath lenticels after being in a solution of 1 per 1000 for one week, and over the general surface most of the lenticels showed as small brown spots none exceeding 1 mm. diameter.

The cuticle and bloom of apples is therefore as impermeable to acids as it is to metallic poisons. Another point worthy of note is that with the strong acid solutions, the pits are at first pale in colour, the dead tissue softer and more watery, slowly becoming a deeper brown on keeping in air.

Sulphuric Acid.—

Prepared Five Crowns.—One week in solution and three weeks in air.

One per 10,000. Pits confluent and 5 mm. deep.

One per 100,000. Pits smaller, up to 3-4 mm. deep.

One per 1,000,000. Pits smaller, up to 1-3 mm. deep.

One per 50,000,000. Faint superficial browning in some cases.

One per 500,000,000. No perceptible effect.

Prepared Rome Beauties.—In solution one week, and examined after one week in air.

One per 10,000. Pits 1-3 mm. deep.

One per 100,000. From superficial browning to pits 1-2 mm. deep.

One per 1,000,000. All the prepared spots browned superficially, but no distinct pits.

One per 10,000,000. No perceptible effect.

After floating Rome Beauties with a good bloom and normal skin on a solution of 1 per 1000 for 14 days, and then keeping for two weeks in air, a few minute spots appeared on the surface, but no pits, and the pulp remained quite sound. Evidently, therefore, the normal skin of this variety is still more impermeable to sulphuric than it is to nitric acid, and the latter acid being more volatile will more readily enter through the lenticels without necessarily displacing the air in their pores by liquid. This is a point of theoretical rather than of practical importance, since although appreciable traces of nitric acid may be present in the air after thunderstorms, they are hardly in sufficient amount to affect apples during the time they remain present.

The specific resistance to poisons.

The foregoing results show clearly the extraordinary sensitivity of the pulp cells of apples to poisons. They are in fact more sensitive to poisons than any other plant or animal cells hitherto examined. The resistance of the apple as a whole to the external application of poison will depend upon its age, upon the development of the cuticle and bloom, and upon the variety of apple.

In regard to the influence of age, the inherent resistance of the pulp cells progressively decreases as the apple becomes adult. In cool storage the sensitivity of apples to poison is only slightly increased even after five months. After removal from cool storage, however, the sensitivity rapidly increases after the first week or two.

Apples with an unbroken skin and good bloom may be immersed in poisonous solutions of far greater concentration than that required to kill the pulp cells. An adult apple is in fact a most delicate but marvellously well-protected mechanism. The young apple is less well-protected externally, but its pulp cells have a greater inherent resistance to poison. A young apple could, in fact, absorb sufficient poison to kill its cells when adult without being affected at first while it was young.

For the above reasons it is difficult to make exact comparisons between different varieties, but with apples of equal age, the sensitivity of the pulp cells to metallic poisons is in the following order:—Five Crown, Jonathan, Scarlet Nonpareil, Rokewood, Rome Beauty, Yates' Pippin[1], the last-named being much more resistant than the first. There are, however, naturally some exceptions with specific poisons. Thus Rokewoods and Yates' Pippins appear to be less sensitive to lead than Jonathans, and Yates' Pippins were also much less sensitive to copper sulphate. Similarly brucine nitrate was apparently slightly more poisonous to Rome Beauties than strychnine sulphate, but with Five Crowns this was reversed. Rokewood and Rome Beauties showed almost exactly the same sensitivity to citric acid, but not to more active poisons.

A very interesting fact noticed was that as a general rule with minimal concentrations of all poisons, the poisonous action usually disappears first from the more exposed side of the apples as indicated by the colour of the skin. Thus the prepared

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1 Yates Pippin appears to be comparatively immune to bitter pit under natural conditions, at least Dr. White found no apples of this variety affected by it in any of the orchards examined by her.

areas on the paler shaded side may still show faint but distinct signs of poisoning, while those on the red side are quite unaffected. This greater resistance of the superficial pulp cells on the exposed side to poison is not due to the cuticle or bloom since they have been removed prior to the test. There is no perceptible difference in the thickness of the cell walls, but the pulp cells in some cases at least were slightly smaller on the exposed side, and hence presumably contained a relatively greater bulk of protoplasm to be poisoned. It can often be noticed that when an apple is strongly exposed on one side, and shaded on the other, it develops a greater radius on the shaded side. Apparently this is partly, at least, due to the cells becoming larger on that side. Dr. White noticed that bitter pit was commoner on the shady side of the fruit or tree than on the exposed side, and suggested that the moisture would persist longer on the shaded side and so favour the entry of spray poisons. Apparently, however, the exposed side of an apple actually retains a greater resistance to poisons than does the shaded side.

The influence of ionization and of the respective ions.

In the case of all compounds which undergo dissociation when dissolved in water, the poisonous action is the result of the joint action and non-action of the different ions and of the undissociated molecules of the compound. A salt may be poisonous on account of either the basic or the acidic radicle composing it being poisonous, or both of them[1]. Further, if one of the ions is much more poisonous than the other, or than the undissociated salt, then increasing dilution, since it increases the relative omount of dissociation, will not produce a decreased poisonous action in direct but in decreasing ratio to the dilution. To some extent this may explain the excessive dilutions at which such salts as mercury, copper, etc., may remain poisonous. More exact comparisons can only be made by calculating the poisonous concentrations in terms of the molecular weights of the

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1 For literature see Pfeffer's Physiology, vol. ii., p. 273. (Eng. Trans.)

different compounds. This is done in the following table, in which the first column gives the poison used, the second the variety of apple, the third the duration of exposure to the poisonous solution, the fourth the lowest concentration at which signs were shown of any distinct poisoning of the pulp cells in prepared apples, and the fifth this concentration in molecular equivalents, so that a comparison is possible between the poisonous action of equal number of molecules:—

Poison.	Kind of apple.	Exposure in days.	Poisonous limit* in grams per c.c. of water	Numerical Poisonous action on an equimolecular basis.
Mercuric chloride	Jonathan	7	1 per 1,000,000,000	271,000
Mercuric chloride	Yates' Pippin	7	1 per 100,000,000	27,100
Copper sulphate	Jonathan	7	1 per 100,000,000	24,900
Copper sulphate	Yates' Pippin	7	1 per 1,000,000	249
Lead nitrate	Jonathan	7	1 per 25,000,000	8275
Lead nitrate	Yates' Pippin	7	1 per 2,500,000	827
Lead nitrate	Rokewood	7	1 per 10,000,000	3310
Lead nitrate	Young Jonathans	7	1 per 1,000,000	331
Lead arsenate	(P. O'Gara)	on tree	1 per 13,000,000**	5876
Gold chloride	Yates' Pippin	4	1 per 10,000,000	3020
Zinc sulphate	Five Crown	$3\frac{1}{2}$	1 per 2,500,000	717
Zinc sulphate	Rome Beauty	7	1 per 1,000,000	287
Arsenic (trioxide?)	Jonathan	4	1 per 500,000***	98.8
Magnesium sulphate	Five Crown	$3\frac{1}{2}$	1 per 100,000	24.6
Magnesium sulphate	Rome Beauty	7	1 per 10,000	2.4
Magnesium sulphate	Yates' Pippin	7	1 per 5,000	1.2
Barium chlorate	Jonathan	7	1 per 100,000	30.3
Barium chlorate	Rome Beauty	7	1 per 10,000	3.0
Barium chlorate	Yates' Pippin	7	1 per 10,000	3.0
Calcium hydrate	Jonathan	7	1 per 5,000	0.37
Potassium chlorate	Jonathan	7	1 per 10,000	1.2
Potassium chlorate	Rome Beauty	7	1 per 10,000	1.2
Formaldehyde	Yates' Pippin	3	1 per 10,000	1
Chloroform	Jonathan	7	1 c.c. per 100,000	7.9

*The concentration given is that at which quite a distinct poisonous action is still perceptible, and not that at which it is just fading out, one litre of solution being used to each apple.

**Estimated from P. O'Gara's analyses of the percentage of arsenic in dead pit tissue produced by arsenate of lead.

***Estimated from percentage of arsenic present in strongly poisoned tissue. The real limit is probably much lower.

Poison.	Kind of apple.	Exposure in days.	Poisonous limit* in grams per c.c. of water.	Numerical Poisonous action on an equi-molecular basis.
Brucine	Rome Beauty	7	1 per 25,000	11.9
Brucine nitrate	Five Crown	3½	1 per 1,000,000	476
Brucine nitrate	Rome Beauty	7	1 per 100,000	47.6
Morphine sulphate	Five Crown	3½	1 per 1,000,000	381
Strychnine sulphate	Five Crown	3½	1 per 1,000,000	430
Strychnine sulphate	Rome Beauty	7	1 per 1,000,000	430
Strychnine sulphate	Yates' Pippin	7	1 per 25,000	10.8
Atropine sulphate	Five Crown	3½	1 per 1,000,000	385
Oxalic acid	Five Crown	3½	1 per 100,000	9
Oxalic acid	Rome Beauty	7	1 per 100,000	9
Citric acid	Rokewood	7	1 per 100,000	19.2
Citric acid	Rome Beauty	7	1 per 100,000	19.2
Citric acid	Yates' Pippin	7	1 per 10,000	1.9
Nitric acid	Five Crown	3	1 per 1,000,000	63
Nitric acid	Rome Beauty	7	1 per 100,000	6.3
Sulphuric acid	Five Crown	7	1c.c. per 10,000,000	532
Sulphuric acid	Rome Beauty	7	1c.c. per 1,000,000	53
Ammonia	Jonathan	7	1c.c. per 10,000	0.4

Generally summarising the means of the foregoing results, the substances tested may be grouped in the following order as regards their toxicity to adult apples. Mercuric chloride, copper sulphate, lead nitrate, gold chloride, zinc sulphate, sulphuric acid, the sulphates and nitrates of brucine, strychnine, atropine and morphine, nitric acid, barium chlorate, magnesium sulphate, citric acid, oxalic acid, chloroform, potassium chlorate, formaldehyde, ammonia, calcium hydrate. It follows also from the table that the poisonous action of the mercuric, cupric, lead and gold salts is due almost solely to their basic components. The comparison between barium and potassium chlorate shows that barium as a base is distinctly poisonous to apples, whereas the poisonous action of magnesium sulphate is largely due to its dissociated sulphate ions. To a lesser extent the same is true for the salts of alkaloids, but the bases in this case do appear to have themselves an inherent and fairly pronounced poisonous action. Strychnine was much more poisonous to Rome Beauties than the other alkaloids. The relatively feeble poisonous action of oxalic acid as compared with sulphuric acid may be partly due to the lesser dissociation of its hydrogen ions, and partly to its displacing bases less readily in the protoplasm of the pulp cells than sulphuric acid. Either the latter reason or a greater ease of penetration may explain why citric acid appears to be more poisonous than oxalic acid, or the oxalic acid may be precipitated to some extent without injury resulting. The almost innocuous character of calcium hydrate (limewater) is due to the carbon dioxide from the respiring pulp cells precipitating the calcium ions as chalk.

It is worth noting in this respect that the order of toxicity in the list above differs very widely on a quantitative molecular basis from what it would be in the case of an ordinary animal. This is especially shown in the relative positions of the alkaloids, and heavy metals, in the position of sulphuric acid, in citric acid preceding oxalic, and in magnesium sulphate preceding formaldehyde and ammonia. In animals, however, any poison which injures one organ may cause the death of the whole organism, whereas in plants, the effects of poisoning may be quite localised, and the death of a leaf by poisoning may not affect the plant more than the mere removal of the leaf would do, while the death of a fruit killed by poison may actually be of benefit to the mother plant, and economise its food supplies.

Naturally if both kation and anion are poisonous, a salt may be more poisonous than either of its ions applied singly. Thus brucine is sparingly soluble in water, but a solution in water only showed distinct signs of being poisonous to the pulp cells of Jonathan and Rome Beauty apples in a concentration of 1 in 25,000, doubtfully so in the case of 1 per 100,000, and none at all in the case of 1 per 1,000,000. The sulphate was, however, feebly but distinctly poisonous down to concentrations of 1 per 100,000.

Further, a mixture of poisonous salts may be less poisonous than either salt singly. In some cases the reason for this is obvious. For instance, a mixture of 303 cc. of 1 per 100,000 barium chlorate and 246 cc. of magnesium sulphate 1 per 100,000, is slightly less poisonous than the last solution applied singly. This is simply because the barium is precipitated as the insoluble sulphate, and the chlorate ions of the magnesium salt remaining are less toxic than the sulphate ions.

In the same way the presence of a sulphate in the cells will protect them against the toxic action of barium. Thus prepared apples floated for two days on a 1 per 10,000 solution of potassium sulphate and then on a 1 per 10,000 solution of barium chlorate for five days, showed only doubtful or no signs of poisoning, whereas after one week on 1 per 10,000 of potassium sulphate slight, and after one week on 1 per 10,000 of barium chlorate distinct, signs of poisoning were produced.

Even where no precipitation takes place, two salts jointly may not exercise any greater toxic action than either salt singly when in considerable dilution, although the total concentration is doubled with the mixtures of the two salts. This is well shown by the following results:—

Prepared Rome Beauties.—One week on solution, and examined after one week.

Barium chlorate, 1 per 10,000. From superficial browning to pits 1 mm. deep.

Barium chlorate 1 per 10,000, and potassium chlorate 1 per 10,000. From superficial browning to pits 1 mm. deep.

Potassium chlorate, 1 per 10,000. Superficial browning to pits 0.5 mm. deep.

Potassium chlorate 1 per 10,000, and magnesium sulphate 1 per 10,000. Superficial browning to pits 0.5 mm. deep.

The addition of a poisonous anion to a dilute toxic solution may even decrease the poisonous action of the mixture if it diminishes the amount of dissociation of a highly toxic kation. This is probably the explanation of the following results in which the addition of citric acid diminished the toxicity of copper sulphate, and the addition of sulphuric acid to copper sulphate produced no increase, but if anything a slight decrease of toxicity. The materials and times were as in the experiments above[1].

Copper sulphate, 1 per 1,000,000. Pits 1-2 mm. deep.

Copper sulphate 1 per 1,000,000, and sulphuric acid 1 per 2,000,000. Pits mostly just over 1 mm. deep.

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1 Mr. Rivett informs me that the decrease in the concentration of copper ions would be much greater in the presence of citric acid than in the presence of an equivalent amount of sulphuric acid. The decrease is in fact so great that no copper hydroxide can be precipitated by caustic alkali.

Copper sulphate 1 per 1,000,000, and citric acid 10 per 1,000,000. From slight superficial browning to pits 1 mm. deep.

The nature of toxicity.

It is generally assumed that the toxicity of the heavy metals is due to their combining with the proteids of protoplasm, and that they are toxic in great dilution because the protoplasm has the power of absorbing and accumulating them in greater concentration than in the liquid outside. To some extent this is true, but nevertheless the foregoing experiments have shown that pulp cells poisoned by mercuric chloride may not contain this poison in a concentration of more than 1 in 30 to 100 millions, and possibly even less. It must be remembered, however, that the adult pulp cells of apples have only a thin layer of protoplasm, and that if this is killed at any point, however small, the death of the whole cell follows for physical reasons, in much the same way that a balloon collapses when pricked by a pin.

Certain cases of toxicity appear to be due to the removal by displacement or ionic interchange of the kations of metallic physiological salts present in the cell, such as calcium nucleoproteate. Thus Loew[1] has shown that various substances which precipate calcium (sodium fluoride, potassium oxalate) are poisonous to *Spirogyra*, and apparently act by removing the calcium from the nucleo-protein acids of the nucleus.

Similarly Loeb[2] showed that sodium chloride was poisonous to the eggs of a certain fish, but not when calcium was present in the external solution, and according to Loew[3] this action would be due to the removal of calcium in the one case, and its non-removal in the other. It is possible that the feebly poisonous action of potassium salts might be due to their displacing calcium or magnesium from the protoplasm of the pulp cells of apples, but if so it is difficult to understand why the addition of a potassium salt to a magnesium one does not increase the poisonous action, but if anything has the reverse effect. It may be that since magnesium and potassium are present inside the

1 Bull. Coll. Agric., Tokyo., Imp. Univ., vii. 1906., p. 18.

2 Arch. f. f. ges. Physiol. 107, p. 252., 1905.

3 See also Osborne, Proc. Physiol. Soc. xxxiii, 1905, p. 1. and 1906, xxxiv., p. 84.

cell, the presence of both outside the cell approximates more closely to a physiologically balanced solution, and produces less protoplasmic disturbances than when one alone is present outside the cell. Taking as a definition of a toxic substance that it is one which in relatively small amounts injures or kills the cells in detail, or the living organism as a whole, all such substances may be termed toxic so far as apples are concerned, independently of what the details of their action may be.

Taking the poisonous dose of mercuric chloride as compared with the body weight of a man to be 1 per 100,000, the pulp cells of apples are certainly 100, and possibly much more than 1000, times as sensitive as man, comparing the quantity required to poison a given amount of material. With alkaloids the reverse is the case. Curiously enough, formaldehyde is less poisonous to apples than almost any other substance tried, and is certainly far less relatively poisonous to apples than it is to animals. No evidence of any polymerization could be detected, so that evidently the adult pulp cells have developed a special resistance to a substance which they themselves produce when young and assimilating.

The action of gases and volatile substances.

The possibility of poisons in volatile form reaching apples needs consideration, and accordingly a variety of substances more or less likely to be present in air were tried.

Ammonia.—Strong ammonia vapour rapidly blackens the skin of sound Jonathan apples, more dilute vapours brown the surface of Jonathans and Sturmer Pippin, and produce small pits within three days, mainly beneath breathing pores. For quantitative estimation of the toxicity, solutions of ammonia (0.88 sp. gr.) in varying amounts of water were used. Thus with Jonathan with a sound, uninjured skin after 1 week in 1 c.c. of 0.88 in 200 cc. of water, within a week numerous brown spots 1-5 mm. diameter, and from superficial to 3-4 mm. deep appeared, but in a litre of 1 per 2000 cc., no spots or pits formed even after one week's immersal and five weeks in air.

Prepared Jonathans.— One week in solution, and examined one week after removal from solution.

One c.c. per 2000. Pits from superficial to 2-3 mm. deep.
One c.c. per 20,000· Superficial browning to pits 1 mm. deep.
One c.c. per 100,000 No effect to slight superficial browning.
One c.c. per 1,000,000. No perceptible effect at any time.

Ozone.—Each apple (Jonathans and Sturmer Pippins) was placed in a 1½ litre gas cylinder, charged with ozone by the silent discharge of high-tension electricity (10-inch spark coil) for two minutes daily for three days[1]. No perceptible effect was produced except that many of the breathing pores became more prominent.

The experiment was then repeated, charging three times daily for five minutes at a time for three days (a total of 45 minutes charging). The apples were covered with small spots and pits. The latter in one week were 1-2 mm. diameter and 2-3 mm. deep. These were almost entirely developed under breathing pores, but a few formed under the unbroken skin. Closely similar effects were produced by one charging of an hour's duration. The air was neither acid nor alkaline, and the ozone rapidly disappeared in contact with apples, no trace being detectable with starch and potassium iodide paper after 10 to 24 hours. Neither ozone nor ammonia are, however, likely to be present under natural conditions in sufficient amount, and for a sufficient length of time, to affect apples, except under most unusual circumstances.

Iodine.—A solid substance which is volatile, may, however, affect apples at some distance. Thus a Jonathan apple was placed in a closed 1½ litre gas cylinder one foot distance from 5 milligrams of iodine. In five days the iodine had sublimed, and in seven days the apple was covered with small dark spots. No smell of iodine was perceptible, but starch paper placed on the apple in the cylinder was tinged faint blue in 20 minutes. After two weeks in air, some of the spots had enlarged to pits 1 to 2 mm. diameter, and 1 mm. deep.

On repeating the same experiment, keeping the cylinder closed for 14 days, two weeks later many of the spots developed into pits 1 to 2 mm. deep, and a few larger discoloured areas developed

1 The apples were removed during each charging, since the silent discharge if long continued, produces brown spots and areas of variable depth, particularly if the apples touch the glass, or from the point of suspension if this affords a ready path for the current.

beneath or between breathing pores to a depth of 3 to 4 millimetres. About the same effect was produced by placing apples six inches away from 40 milligrams of iodine for two days, but the spots appeared much sooner.

In the case of other volatile substances such as prussic acid, the diffusion is so rapid that the poisoning of the pulp takes place generally unless the correct time of exposure is found to produce pits. Thus one and two days' exposure to diluted hydrocyanic acid killed the pulp throughout. After six hours' exposure irregular brown areas of pulp appeared beneath the skin, some becoming sunken and pit-like, and a few brown patches radiated from the centre, unconnected with the surface.

Chloroform.—Normal Jonathan apples kept in a 1 per 1000 solution for three days, developed numerous large and small spots and pits, and in many cases a white "efflorescence" appeared on the skin, due to the partial solution of the bloom. In 1 per 100,000 even after one week no pits or spots were found, although a faint smell of chloroform was perceptible on opening the cylinder.

Prepared Jonathan Apples.—Examined after two weeks in air.

One per 1000 (three days). Large, deep pits, with typical brown, spongy, shrivelled bitter pit cells.

One per 10,000 (one week). Pits 2-5 mm. deep.

One per 100,000 (one week). Pits 1-3 mm. deep.

One per 1,000,000 (one week). No perceptible signs of poisoning.

Arseniuretted Hydrogen.—Whenever arsenic compounds are in contact with organic matter, and particularly in the presence of certain fungi, there is a possibility of the evolution of arseniuretted hydrogen gas. The conditions for the development of minute traces of arseniuretted hydrogen gas, are present in any orchard heavily sprayed with arsenical compounds, particularly during close, moist weather.

Normal Jonathan apples were exposed each in a 1500 cc. gas cylinder to the arseniuretted hydrogen from 1 gram As_2O_3, and after keeping in air two weeks to allow all free gas to escape, forwarded for analysis[1].

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1 During Mr. Wilkinson's absence these tests were performed by Mr. Willgerodt, using the very delicate Gutzeit test for arsenic.

Six Hours' Exposure.—After two weeks numerous brown spots and deep pits of variable size.

Customs' Analyst:—"No arsenic detected, but the extract frothed over owing to a mishap, before the test was quite completed. If any arsenic was present it was less than in the following cases."

One Day's Exposure.—General browning and two large dead areas 2 to 4 mm. deep.

Customs' Analyst:—"The total arsenic content of the apple 'Q' was equal to about 0.05 mg. arsenious acid (As_2O_3), or 1-3300th of a grain."

Two Days' Exposure.—Whole superficial layer soft, brown and dead to a depth of 5 to 10 mm., a little dead tissue also radiating from the core into the outer pulp.

Customs' Analyst:—"Apple 'R' contained a slightly larger amount of arsenious acid than apple 'Q.'"

Four days' Exposure.—Same effect as before, but more pronounced. The volume of the apple was 104 cc., and approximately half of the pulp was dead.

Customs' Analyst:—"The apple 'S' contained the largest amount, equal to about 0.10 to 0.15 mg. As_2O_3 (1-1600th to 1-1000th of a grain)."

The arsenic was therefore in this case in a concentration of approximately 1 in 300,000 to 1 in 500,000.

In another experiment a Jonathan apple was exposed to the AsH_3 from 5 mgms. of As_2O_3 for one week. After three weeks in air, the skin was browned all over, and a few shallow pits developed. Even here the Federal Analyst was able to detect distinct indications of the presence of arsenic, although apparently hardly in accurately measurable quantity.

Perhaps the most important point here is that apples appear to have the power of decomposing arseniuretted hydrogen and retaining its arsenic. The gas itself is only sparingly soluble in water, and after two to three weeks in air, it is hardly likely that any of the gas could still be present as such in the apples particularly when the arsenic is only present in the skin, as in the first and last experiments. Hence very minute traces of arseniuretted hydrogen could be picked up and accumulated by apples until in time the exceedingly small amount necessary to poison the pulp cells was reached, which would in this case be at points under or near to the breathing pores or to the centre of the fruit.

Part II.

The Problem of Bitter Pit.

According to C. P. Lounsbury (Agricultural Journal, Cape of Good Hope, 1910, page 150), this disease is a physiological one. The spots develop while the apples are on the trees, and also in apparently healthy fruit after storage. Certain varieties are more immune, particularly those from colonial seedlings. Hence he considers that by the selection of resistant varieties, the disease may be partly controlled.

I. B. P. Evans (Transvaal Department of Agriculture, Technical Bulletin I.) found that all imported varieties were more or less subject to this disease, but that two native seedlings were practically immune. The spots arise in close connection with the vascular bundles where the excess pressure bursts the pulp cells. The oxygen with the enzymes acts on the tannin, producing dark coloured oxy-compounds, and drastic action is inhibited. The bursting pressure is produced during the night stoppage of transpiration.

Cases of cells bursting during development are known to occur with pollen tubes and root hairs, but only when grown under abnormal artificial conditions, and here we are dealing with unicellular structures. Pole-Evans has overlooked the fact that in the pulp cells of the apple we have a tissue in which the cell walls press against each other, and hence are under compression instead of tension at the points of contact. Further, a localised increase of pressure by distending the walls would diminish or obliterate the air spaces, and bursting would only occur when the breaking strain was reached before the expansion was completed. In plant tissues this appears only to occur when preceded by a softening of the cell wall, of which there is no sign in bitter pit.

Further, puncturing young apples and half-grown apples on the tree with fine sterilised needles so as to rupture some of the pulp cells, may result in the production of flat or crater-shaped more or less corky superficial scars, but never causes an appearance of bitter pit in Jonathans, Five Crowns or Scarlet Nonpareils.

Pole-Evans' theory is apparently based upon the idea that the stoppage of transpiration produces a sudden increase of pressure, just as occurs when a stream of water flowing through a hose pipe is blocked at the orifice. This is of course a fundamental misconception, since the osmotic pressure depends upon the osmotic concentration of the cell sap which the plant is able to control, and as soon as the hydrostatic pressure in the cell balances the osmotic pressure, further absorption of water ceases.

Pole-Evans' theory was based upon the simple observation, which is quite correct, that sections of bitter pit tissue commonly show broken cell walls. The same is also shown in artificial bitter pit tissue produced by poisoning. As the cells die and shrivel, the walls become brittle, and being thin are easily broken either by the general contraction or by the tearing of the razor during the section cutting. Natural bitter pits treated with dilute potash and carefully teased out may sometimes not show a single broken cell-wall, and the more carefully sections are cut the fewer are the broken cell walls. Further, when broken cell walls appear naturally in artificial bitter pits, they usually occur at the boundary of the pit tissue and normal pulp cells, the adherence of the former to the skin causing the breaks when the shrivelling is pronounced. In both natural and artificial bitter pits, any breaking of the cell walls follows instead of precedes the formation of the pit.

Dr. White then investigated the enzymes of apples affected by bitter pit, and from her observations, came to the conclusion that the non-solution of the starch grains which usually accompanies bitter pit could only be explained as a symptom of local poisoning. She was able to detect the presence of lead in the bitter pit tissue of apples from an orchard which had been heavily sprayed with arsenate of lead[1]. The fact that bitter pit may also occur in orchards which have never been sprayed does not affect the theory that bitter pit is not a disease at all, but a symptom of local poisoning. The two most trenchant

1 Proc. Roy. Soc. of Victoria, 1911, p.1.

criticisms made to contest the statement that bitter pit could ever be due to poisonous spraying materials were—(a), that if so, it would occur most in the cup at the stalk end of the fruit, and (b), that in the apples examined by Dr. White the lead might have entered after the bitter pits were formed. In regard to (a) the answer is simple. The stalk end is at first convex, and the cup only develops as the fruit becomes adult. It is then almost without exception covered by a more or less corky, protective, impermeable layer, and has few or no breathing pores through which poisons could enter. The reply to (b) is given by the experiments already detailed and to follow.

The fact that poisonous sprays may affect the foliage is well known, and it would be in the highest degree remarkable if under no circumstances were any effects exercised on the fruits. In 1902 Bain investigated the injurious action of copper fungicides on peach foliage[1]. He found that penetration did not necessarily take place through the stomata, and that the injury might appear at some distance from the point where the drop of spray material (Bordeaux mixture) was applied. Further, he found that at first the production of starch was increased, and that later signs of injury or death were shown. If the amount of poison is sufficient, the dead cells will retain undissolved starch grains. In other words, we have here a "bitter pit" of foliage directly produced by poisonous sprays. Bain also found that seedling apples were more sensitive to poisons than seedling peaches, and were rapidly killed when a solution containing 0.0000075 gram molecules of copper sulphate per litre was applied to their roots.

G. Stone (Massachusetts' Station Report), found that during 1909 spraying with Bordeaux mixture caused noticeable injury to apple foliage and fruit, and that arsenate of lead spraying caused foliage burning, especially on plum trees, but also on maple and beech.

W. N. Scott (Phytopathology, 1911, page 32) found that a fruit spot of apples, particularly of Jonathan and Esopus, which develops usually after picking, is dark brown, usually circular, from $\frac{1}{4}$ to $\frac{1}{8}$ inch or less in diameter, and slightly sunken with a lenticel in the centre of each spot. It has been observed in

1 Agric. Exper. Station, Univ. of Tennessee, vol. xv., p. 21.

apples from Iowa, Missouri, Virginia, New York, Oregon and Washington. The cause is uncertain, but it is suspected to be due to injury by arsenate of lead used in spraying. Low temperatures retard or prevent its development.

P. O'Gara ("Better Fruit," 1911, p. 28) writes "On the spotting of apples by arsenate of lead." The spots are confined to the epidermal and sub-epidermal cells. An unsprayed orchard with neglected trees did not develop a single spotted fruit except where the trees were sprayed with a soluble arsenical poison. The greatest amount of injury was found in trees in the very best condition, growing prize fruit and regularly treated. Analyses of 10 gramme samples of the epidermal and sub-epidermal tissues showed that the black and red spots contained from 0.03 to 0.05 milligramme, while the apparently sound skins showed 0.025 milligramme of arsenic. One analysis of very badly spotted apples showed a quantity of arsenic equal to 0.3 mg. of As_2O_5(0.005 grain). There is no danger from eating such apples, as at least 5 milligrammes are required to produce a marked effect. The author advises the use of at least one pound of unslaked lime with each pound of lead arsenate.

In view of the foregoing results direct tests were made with various spraying materials.

Arsenate of Lead.—That this and other spraying materials termed "insoluble" are not absolutely so was shown by the fact that a clear saturated solution from Sherwin-Williams' new process arsenate of lead contained 0.06 of dissolved solids.

Jonathan apples rubbed with the solid, then wiped clean and wetted daily with water, developed numerous black spots at the lenticels, but even after eight weeks none exceeded 1 to 2 mm. diameter and 0.5 mm. depth, and the pits were no larger even when minute punctures not exceeding 0.2 mm. diameter were made in the skin prior to rubbing with arsenate of lead. If fragments of the cuticle 1 mm. square were removed, dark, circular brown pits formed, increasing to 2 to 4 mm. diameter and 1 to 3 mm. deep in eight weeks. Prepared apples placed for one week in a clear filtered solution from 5 grams of arsenate of lead in 1 litre of water, filtered after standing, showed in three weeks well-developed pits 1-2 mm. deep beneath each prepared spot. With a similar solution from Paris Green the pits were 3-4 mm. deep, but with solutions from lead chromate and London Purple, only a superficial browning and a few pits not exceeding 1 mm. depth were formed.

Sound apples with a normal skin floated for one week on clear saturated solutions from arsenate of lead, lead chromate, London Purple and Paris Green, allowed to stand, decanted, and then filtered, showed after three weeks in air from a few to numerous small superficial spots, but no pits, and the pulp was wholly sound. The above tests were done with Jonathan, Yates' Pippin and Scarlet Nonpareil apples.

Evidently, therefore, all these substances are sufficiently poisonous to poison the pulp cells when they gain entry, but are unable (in solution in appreciable amount) to directly penetrate the skin of a sound adult apple with a well-developed cuticle and bloom.

Bain (l.c. p. 43) found that the injurious action of Bordeaux mixture and copper hydrate on peach leaves was greatly decreased in the presence of free lime or calcium carbonate. Hence, the influence of these substances by themselves was tried. Apples floated on lime water had chalk deposited on the skin, but even after one week showed no signs of injury, and the chalk readily rubbed off.

Prepared apples placed in saturated lime water for one week formed shallow brown pits beneath each prepared spot on keeping, in a 1-10th saturated solution only a superficial browning appeared, and a 1-100th saturated solution produced no perceptible effect. Prepared apples placed in a clear saturated solution of lead arsenate, to which an equal bulk of 1-100th saturated lime water was added, showed no signs of poisoning, or only very faint superficial browning even after one week.

It is quite clear therefore that the addition of free lime to arsenate of lead diminishes its toxicity, and would probably also make the same quantity go further in practice. Whether the mixture would be equally effective and firmly adherent could only be determined by field trials.

Other spraying materials.

Apples rubbed on one side with emulsified spraying oil (Emulsi-A-01), developed in one to three weeks several circular pits 1-3 mm. diameter and depth beneath some of the lenticels, and a few larger irregular, superficial, brown, discoloured patches on this side, but not on the other. When rubbed with red oil, no effect was produced. Apples are not sensitive to all poisons, antiseptics or disinfectants. Thus when rubbed strongly with iodoform, and then wiped clean, a faint smell of iodoform was perceptible, even after three weeks. A few of the lenticels showed as small superficial brown spots, but no pits were developed, and the pulp remained sound throughout.

Can surface poisoning produce deep seated bitter pit tissue?

In all the experiments on adult apples with liquid or solid poisons, the effects were always produced first at the point where the poison was applied, and thence radiated inwards, but in normal bitter pit, the dead tissue may sometimes be deap-seated without any apparent connection with the exterior. There is, however, a possibility that poisons might enter in too small amount to poison the resistant young apple cells, and later when more deep-seated, might poison the adult pulp cells as soon as their resistance had decreased sufficiently.

As a matter of fact apples appear to grow more peripherally than centrally after setting. Thus in Jonathan and Five Crown apples of 1 and 1.1 cm. radius, the diameter of the central core was 0.4 cm., giving ratios of 2.5 and 2.75, whereas in adult apples the relationships were:—

Radius of apple.	Radius of core of loculi.	Ratio.
3	0.8	3.75
3.7	1.2	3.03

Hence poison originally near the surface might become deep seated as the apple grew to adult size, in just the same way that substances at first on the surface may in time be imbedded deeply in growing wood. In the case of the apple, however, we should expect to find in most cases at least, a more or less prominent track up to the surface, and probably many or most of those cases where so such track is perceptible, and where the bitter pit tissue is entirely deep seated, are the result of the absorption of minute traces of poisons through the roots, or less probably, of volatile substances from the air.

To determine this, young apples three weeks old, as yet with no starch, were finely punctured just through the skin, and rubbed over with arsenate of lead. At three months all those remaining on the tree showed deep brown spots at or below the point of injury, and in some cases it was difficult to trace any connection with the injury on the skin. The dead tissue in this case contained few starch grains as compared with the normal pulp, but this was because most of the cells were killed before the deposition of starch grains had begun in the pulp tissue.

The starch grains of bitter pit.

One of the characteristic features of bitter pit is stated to be that cells filled with starch grains occur in the dead tissue when none are present in the healthy pulp cells. Out of some hundreds of artificial bitter pits examined which had been produced by different poisons, practically in every case where the pits were sufficiently large, occasional isolated cells packed with starch grains could be detected. At first this appeared to point to a condensation of starch in the cells as they were gradually killed by minute traces of poison. Prepared adult apples floated on dilute non-poisonous solutions of glycerine, sugar and formaldehyde, showed no perceptible formation and accumulation of starch in the pulp cells under the prepared spots, so that apparently the leucoplastids in adult pulp cells, which still appear to be present in some cases particularly near the surface, appear to have lost the power of producing starch. Further investigation showed that the presence of occasional cells, or even groups of cells living but packed with starch grains, is a common and almost universal occurrence in ripe apples. The sound pulp of more than 200 ripe apples of the kinds mentioned above was examined for starch, and in 95 per cent. with positive results. These starch cells are always sparsely scattered in sound apples, and are least abundant in Five Crowns and most in Jonathans. The individual starch cells when present are, however, packed with starch grains showing no signs of solution. In Five Crowns there are rarely more than 0 to 6 such cells in a preparation 1 cm. diameter, and 1 to three cells thick, whereas in Jonathans there may be from 0 to 50 such cells present in the same amount of sound, living pulp. These cells show no other signs of injury or death, their protoplasm appeared to be living, and when tested on carefully made preparations was plasmolysable. A sound, ripe Jonathan apple, free of all external signs of bitter pit, may, therefore, contain some thousands of living but starch-bearing cells. So far as the apples examined were concerned, the non-solution of the starch grains in occasional scattered pulp cells is not an abnormal but a normal phenomenon.

The non-solution of the starch grains.

The question at once arises, Why do the starch grains in occasional cells of otherwise sound apples and in numerous cells in ordinary bitter pit tissue, remain undissolved during ripening?

In the first place it must be noted that natural bitter pit is not accompanied by cells with undissolved starch grains if it appears or is produced in young apples before the starch grains have appeared in the general pulp. Such apples usually fall off without ripening, probably as the result of excessive poisoning[1]. Secondly, when it develops late after storage, there may be no more starch grains in the dead tissue than in the general pulp. Thus Jonathan, Sturmer Pippin and Scarlet Nonpareil apples, which had been heavily sprayed during development, were picked over, and all bitter pit apples removed. An abundance of starch grains was present in the bitter pit tissue in every case. The sound apples were kept in cool storage for four months. Many of them developed large, deep bitter pits, almost every one of which was beneath a breathing pore. Occasional starch containing cells were present in the dead tissue, but not perceptibly more than in the healthy pulp tissue.

The non-solution of starch grains in living pulp cells can only be due to the following factors:—(1) the diastase ferment which

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1 This may be the reason for the fact that so many young fertilized apples often fall from apple trees for no apparent adequate reason.

converts the starch into sugar during ripening is not developed; (2) the cell contains an excess of the product of the diastase reaction, sugar; (3) the starch grains are composed of some special insoluble form of starch; or (4) some poison is present in sufficient amount to inhibit the action of the ferment, without necessarily killing the cells until their power of toxic resistance has fallen to a sufficiently low ebb.

Living pulp cells with undissolved starch grains were soaked in Fehling's solution for half-an-hour, and warmed. Doubtful or no traces of reducing sugar could be detected in them. The same negative result was given after previous warming with dilute sulphuric acid. If they contain any sugar at all, they contain less than ordinary pulp cells. Further, they do not appear to be either constantly more or less plasmolysable than ordinary pulp cells. Possibly they contain some substance in solution which makes up for the absence of sugar[1]. Indeed, if this were not so they would be crushed and flattened during development by the surrounding cells, whereas in adult apples these living starch-bearing cells, though frequently smaller, are often quite as big as the ordinary pulp cells. In any case the non-solution of the starch grains is not due to the cells affected containing an excess of sugar.

In regard to the diastase ferment the following experiment was performed. Two adult prepared Jonathan apples in which small bitter pits had been produced by 1 per 500,000 and 1 per 2,500,000 solutions of lead nitrate, and two ripe normal apples were kept in all for 10 weeks in air at room temperature. Occasional cells with uncorroded starch grains were still present in the sound pulp of all four, and in the dead pit tissue of two of them. The peel and dead pit tissue was then removed, and the pounded pulp of each extracted for diastase[2]. The filtered precipitate from each was divided into two parts, to each of which starch solution was added, and one, the control, was immediately boiled. Both were then kept warm for some hours, and then tested for reducing sugar.

1 This may lead to a vicious cycle. Thus the presence of an infinitesimal trace of poison may delay the solution of starch and cause the cell to contain less sugar than its neighbour. It may maintain its osmotic pressure by absorbing salts from them. If these are at all poisonous, the poisoning becomes more severe and may lead to death, some cells being thus sacrificed as poison traps to preserve the rest.

2 These tests were performed by Dr. White.

	Weight of pulp.	Reduction of Fehling's.
1. Normal apple -	66 grams -	Slight
2. Pitted apple -	65 grams -	Slight
3. Pitted apple -	59 grams -	Very faint
4. Normal apple -	74 grams -	Very faint

Distinct traces of diastase were present in all four cases, and although the amount is small, it is impossible that the starch grains could have remained for 10 weeks without any signs of corrosion, unless something was present which inhibited the diastatic action. Nor is it possible that diastase was present only in the pulp cells without starch grains, for it is well known that diastase can diffuse from cell to cell[1], and the starch-bearing cells were scattered throughout the pulp tissue.

That there is nothing peculiar in the undissolved starch grains found in normal apparently healthy apples was found by microchemical examination, with the aid of iodine, potash, heat and polarised light. Nevertheless it was not found possible to dissolve them by the aid of solutions of commercial diastase, freshly-prepared barley diastase or "ptyalin," even after applying fresh diastase solutions daily for a week. Thin slices of the pulp were then washed repeatedly with dilute ammonia, dilute hydrochloric acid and water. The starch grains in the starch cells dissolved partly or entirely after three to seven days in freshly-prepared barley diastase, but even then some cells could still be found with starch grains apparently entirely unaffected. If bacteria or fungi were allowed to develop freely, all the starch grains were dissolved in 3 to 14 days, and by the latter time the pulp cells separate from each other, but may remain with unbroken walls for a very long time in the absence of cellulose bacteria. The starch grains may dissolve before fungal hyphae reach them, and form at first an irregular amorphous faintly blue-staining mass, or a granular débris in the cells which originally contained clusters of well-defined starch grains.

Similar results were obtained with natural bitter pit tissue containing abundant starch cells, but here is some cases even after long washing with acid, alkali and water, and in the pre-

1 When fungi are grown on apples containing starch, the grains may begin to dissolve while still an appreciable distance from any fungal hyphae, particularly at any point where the growth of the latter is retarded.

sence of bacteria and fungi, starch cells could still be found after three weeks quite unaffected and undissolved. Apparently the poison inhibiting diastatic action in such cases is a metallic one, firmly adhering to the starch grains. Actual tests showed that the addition of one gram of arsenate of lead to 1000 grams of starch completely protects the starch grains from the corrosive action of ferments, if a thorough mixture is made prior to adding the solution of the ferment, and this although arsenate of lead is a comparatively insoluble poison.

Apples affected with natural bitter pit were inoculated with the hyphae of *Penicillium*, and other undetermined saprophytic fungi, taken from apples undergoing soft rot. The hyphae grew freely in the sound pulp, but usually distinctly avoided the bitter pit areas, at first growing right past or around them before invading them laterally. The starch grains in the bitter pit areas remain partially undissolved long after any starch grains which may be present in healthy pulp cells have disappeared. Obviously the bitter pit areas contain a higher percentage of poison, and the fact that the hyphae will ultimately grown into them is not surprising, since they also grow freely in apples poisoned throughout by mercuric chloride or copper sulphate. Similar results were obtained with artificial bitter pits produced by 1 per 100,000 concentrations of mercury and lead, and to a less extent by copper.

If the poison in question were not the originating cause of the bitter pit, but were produced by the secondary changes during the development of the pit evidenced by the change of colour, by the modification of the cell wall and by the disorganisation of the protoplasmic cell contents, then it should always be repellent to saprophytic fungal hyphae. Artificial bitter pits produced by minimal toxic concentrations of metallic poisons are penetrated by fungal hyphae as readily as, or even more readily than, the healthy pulp tissue or natural pit tissue, and the pits produced by formaldehyde ammonia, potassium and magnesium in moderately toxic concentration, exercise a distinct attraction upon the fungal hyphae. Evidently, therefore, the secondary changes during the development of bitter pit do not result in any noticeable production of distinctly toxic materials. In other words, any poisons present in the pit tissue are the cause of the pit and not the result of it.

The source of the poisons causing bitter pit.

None of the volatile poisons tested appear to be likely causes of bitter pit under natural conditions, and their action would not suffice to explain the permanent character of the poisoning and its localised origin. Nor do apples appear to have any power of autotoxication sufficient to explain the symptoms observed, although they do produce, when young, traces of formaldehyde, which is a poison in excess, but only a relatively feeble one. Its action would, however, be a generalised and a rapidly spreading one, and the tests quoted above showed that it was from 3000 to 300,000 times less poisonous to apples than copper and mercury salts, and, in fact, less poisonous to apples than any of the other poisons tested, with the exception of ammonia and lime water. Evidently, therefore, the poison must be derived from outside the plant, either (1) directly through spray poisons applied to the surface, or (2) by poisons absorbed from the soil through the roots.

The bitter pits starting from the surface usually beneath lenticels are largely due to the entry of spray poisons. If the development takes place late, when the apples are adult, the pit tissue will contain no starch grains, and the same is the case when pits are formed in very young apples. The action of the poison is not to cause the condensation of starch grains, but their non-solution during ripening. Nearly every apple, even if apparently sound, has a few cells in which the requisite toxic concentration has been reached to do this. Such cells only die when either the concentration increases as the result of the continued flow of sap and the evaporation of the surplus water, or when the resistance of the cells with increasing age falls to the toxic limit. This incipient bitter pit which appears to be almost always present in apples, is certainly the result of the absorption of poisons from the soil, and certain food elements (Ca, Mg, K) are capable of acting as poisons when they are not present in their appropriate relative concentrations. The complete and permanent localised arrest of ferment action can only, however, be due to the presence of minute traces of an extremely poisonous heavy metal, such as lead, mercury, copper, etc., or even of gold[1], barium or arsenic compounds.

Poisons derived from the soil may be naturally present, or accumulated from the continued use of poisonous sprays, or from the use of poisons to destroy weeds. Half-a-pound per acre represents a concentration of more than 1 per 100,000,000 per acre of soil one foot deep, and this is the limit of toxicity of copper sulphate to the pulp cells of some varieties of apples. A single Bordeaux spraying may consume more than six lbs. of copper sulphate per acre, most of which ultimately finds its way to the soil. In the case of arsenate of lead, some growers spray 3 to 10 times per season, which represents a considerable addition of poison to the soil, and is furthermore repeated annually. Fortunately plants never absorb more than a small fraction of the total amount of any one substance present in the soil, and although good humus soils are very retentive to metallic poisons, there must be a certain annual removal of poison by drainage. It would, however, be interesting to know whether copper, lead or arsenic can be detected in the soils of long sprayed orchards in amounts approaching or exceeding the limits of their toxicity to the pulp cells of apples.

In order to explain the fact that bitter pit is so much more abundant in well-tended and well-sprayed orchards than in neglected, untended ones, many orchardists have suggested that the ploughing may make the roots grow deeper than normal, and so cause interferences with nutrition. This may be the case if the subsoil contains minute traces of poisons, but otherwise it is not a likely explanation. In the same way the disease has been referred to the use of particular stocks or grafts. The nature of the stock or scion may affect the tendency to a particular disease, just as some organisms are resistant to particular poisons or contagious diseases, but this affords no explanation of the cause of the disease in question. In the same way, the fact that cancer seems sometimes to run in families may show that the tendency of the disease may be to some ex-

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Gold is so readily precipitated in contact with organic matter that the possibility of its absorption from soils seems somewhat remote.

tent hereditary, but gives no explanation or cure for the disease itself. From the purely practical point of view the discovery of a completely resistant stock is highly improbable, and resistant varieties of cultivated plants very frequently have the unfortunate peculiarity that they are economically unprofitable.

SUMMARY.

The ripe pulp cells of apples are more sensitive to various poisons than any other known organisms, the limit of toxic action in the case of mercuric chloride being with a concentration of 1 in 10,000,000,000. The sensitivity to poisons varies considerably in different varieties, and the pulp cells on the shaded side of an apple are more sensitive to poisons than on that grown exposed fully to the sun.

The cuticle and bloom of sound apples are remarkably impermeable to poisonous solutions. Moderately dilute solutions of metallic poisons penetrate through the breathing pores in sufficient amount to produce bitter pits, and yet in such minute quantities as to be incapable of detection, even by very delicate chemical analysis.

When the cuticle or bloom is removed penetration takes place readily. Hence apples should never be wiped or polished until just before they are eaten. Apples have a certain power of accumulating mercury and similar poisons from extremely dilute solutions, but nevertheless, mercuric chloride is poisonous to the pulp cells of apples without any accumulation in concentrations down to 1 per 100,000,000, and possibly even lower. Copper is not quite so poisonous as mercury, and lead much less so, while its action is also slower.

Young apples are more easily penetrated by dissolved poisons than old ones, but their pulp cells are much more resistant. Hence a young apple may absorb sufficient poison to kill a portion of its tissue when adult, without any immediate toxic effect being shown.

Food and other substances occurring normally in the soil may exercise a toxic action when presented singly, but in mixtures their individual poisonous action is decreased.

The poisonous action of copper sulphate and other soluble metallic poisons to apples, may be decreased by the addition of substances which decrease the percentage of free ions, and on these lines it may be possible to modify even such relatively insoluble spray poisons as Bordeaux mixture and arsenate of lead, without appreciably affecting their value as insecticides and fungicides. Zinc arsenite has been recently suggested as being equally effective as an insect poison, and less poisonous to plants than lead arsenate.

Nevertheless since the fungus, *Penicillium*, which is a common cause of wet rot in apples is able to grow on a 20 per cent. solution of copper sulphate, it is 2,000,000,000 times more resistant to copper than the pulp cells of apples, and it is also very much more resistant to mercury salts[1]. In this case "fungicides" will kill apples long before they kill the fungus. Of gases present in the air, ozone, ammonia and nitric acid are able to produce surface pitting in apples, but only in amounts and with lengths of exposure relatively much greater than those presented under ordinary conditions.

Five milligrams of solid iodine placed a foot distant from an apple can cause it to become covered with spots and pits in the course of time.

Apples absorb and accumulate arsenic from arseniuretted hydrogen, a strong poisonous action being exercised when a concentration of 1 of arsenic trioxide per 500,000 of apple pulp is reached. When once within the pulp cells, lead arsenate is poisonous in a concentration of 1 in 13,000,000.

The following results conclusively show that the "disease" of apples known as bitter pit is, strictly speaking, not a "disease" at all, but is a symptom of local poisoning.

Bitter pit symptoms, including the presence of abundant starch grains in dead cells, are frequently shown in the spots appearing on peach and apple foliage, when burning is produced by poisonous sprays. Not only arsenate of lead, but all the other common spray poisons are effective causes of bitter pit in apples. When the entry takes place from the surface of the apple at the close of ripening, the pits are superficial and without starch grains. The usual characteristics of bitter pit are only

1 *Penicillium* can also withstand 15 per cent. of zinc sulphate, 37 per cent. of manganese sulphate, 8 per cent. of ferric sulphate and is also resistant to arsenic, all of which are poisonous to ordinary plants.

shown when a toxic concentration is reached after the cells have become packed with starch. This at first prevents ferment action, and later kills the pulp cells.

The first stage of bitter pit, namely, the presence of occasional cells with starch grains which do not dissolve during ripening in perfectly sound ripe apples, is apparently almost a normal phenomenon whether the apples are from sprayed or unsprayed orchards. This is the result of their extraordinary sensitiveness to traces of poisons.

In such apples and in bitter pit apples from unsprayed orchards the poison must be absorbed from the soil. The relative incidence of bitter pit in sprayed and unsprayed orchards is a matter of great importance. If 90 per cent. occur in sprayed and 10 or less in unsprayed orchards, the immediate problem is to find a substance which will diminish the toxicity of spray poisons to plants, while leaving them equally effective against the insect pests which eat and digest the poisonous spray with the plant tissues.

Among such substances appear to be lime and citric acid, but much more effective ones may be found. So far as the evidence goes at present, bitter pit appears to be much more prevalent in sprayed orchards than in ones which have never been sprayed or had any poison applied to the soil.

On the other hand if only a minority of cases of bitter pit are directly or indirectly due to the use of poisonous sprays, it will be necessary to find what poisons are absorbed from the soil or subsoil, and what correctives can be applied to them. This is a chemical problem of some difficulty, since the traces of poison required to produce bitter pit symptoms in the pulp cells of apples are almost infinitesimally small, and a number of different poisons may be acting on different trees in the same orchard.

On three points, however, it may, I think, be stated with confidence that we are on a solid bed rock of established fact, namely, that bitter pit is, strictly speaking, not a disease at all, but is a symptom of local poisoning produced in the sensitive pulp cells of the apples, that more than one poison may produce it, and that such poisons may be derived from more than one source.